A Method for Assessing Attentional Bias in Anxious Rats

A Senior Honors Thesis

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by

David E. Osher

The Ohio State University
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Project Advisor: Professor Bennet Givens, Department of Psychology

Abstract

A popular theory regarding the etiology of anxiety disorders asserts that they are developed and maintained by an attentional bias towards threat cues. The present study attempts to develop an animal model that parallels current human research on this bias. Adult rats were tested in a 3-choice serial reaction time task with aversive and appetitive signals, and their performance was compared against anxiety scores from elevated plus maze and open field sessions. A bias towards the aversive signal strongly correlated with anxiety scores, suggesting that this methodology could be used for further animal studies investigating their relationship.

Introduction

Attention fuses top-down with bottom-up processes; in other words, it is modulated by executive control as well as salient environmental stimuli. Somewhere in between, attention is also influenced by trait-anxiety. A long established relationship exists between high trait-anxiety and an attentional bias towards threat cues (see Mogg & Bradley, 1998 for a review). This phenomenon was first observed clinically in individuals who suffered from generalized anxiety disorder (GAD), through attentional tasks where performance can be impeded by threat distracters.

One classic demonstration of attentional bias involves a modified Stroop task, where the colored fonts of words have to be named as quickly as possible; some of the words are neutral, such as 'wall' or 'carpet', while others are essentially threat cues, such as 'cancer' or 'murder'. Sufferers of GAD produce a significantly inhibited reaction time to threat words as compared to controls, presumably because they allocate their attentional resources first to the semantic, rather than color content of the words (Mathews & MacLeod, 1985; Mogg, Mathews & Weinman, 1989). Another archetypal experiment is the dot probe detection task, where two words appear directly above and below a fixation point for some amount of time, followed by a dot in one of the two locations. After the dot disappears, subjects must report the location of the dot. Though some of the words are threat cues, at least one of the presented stimuli is always neutral. Here, anxious individuals respond faster than controls when the probe replaces a threat cue, and furthermore, they respond slower when the probe replaces a neutral word whose compliment is a threat cue (MacLeod, Matthews & Tata, 1986; Mogg, Mathews & Eysenck, 1992; Mogg, Bradley & Williams, 1995). Additionally, there exists evidence

that the attentional bias occurs preattentively, outside of awareness. When the modified Stroop task or dot probe detection task is performed under subthreshold conditions, with stimuli presented for 14ms, followed by a mask, the results coincide with suprathreshold conditions: high trait-anxious subjects not only detect threat cues faster than controls, but they also exhibit inhibited task performance when distracted by a threat cue (MacCleod & Rutherford, 1992; Mogg, Bradley, Williams & Mathews, 1993).

Currently, many cognitive theories regarding the etiology of anxiety disorders speculate that this attentional bias plays a key role in the maintenance and development of these disorders (Beck & Clark, 1997; Mogg & Bradley, 1998; Mathews & Mackintosh, 1998). Pharmacological and clinical treatments for anxiety disorders eliminate the bias, and furthermore, current experimental cognitive therapies aimed at ameliorating the attentional bias have lead to significant reductions in anxiety symptoms (Mathews, Mogg, Kentish & Eysenck, 1995; Hazen, Vasey, & Schmidt, 2004). These effects certainly reveal the possibility of an etiological relationship.

The goal of the present study was to develop an animal model for this attentional bias. In turn, this will entail further investigations regarding the biological substrate of the phenomenon, and offer additional insight about the relationship between anxiety and attentional bias. In human research, a cognitive model proposed by Williams, Watts, MacLeod, and Matthews (1997) has been particularly influential to current investigations, and served as the foundation for our methodology as well. Two distinctive information processing stages characterize this model, detailed in Figure 1: an affective decision mechanism (ADM) and a resource allocation mechanism (RAM). The ADM initially encodes the threat value of stimuli; if this value surpasses a threshold determined by the

individual's state anxiety, then the ADM triggers the RAM. The RAM reflects an individual's trait anxiety, which can be described as a disposition toward anxiety states. The RAM assigns attentional resources to stimuli, based on trait anxiety: individuals with high-trait anxiety are more likely to attend to the threatening stimulus, while those with low-trait anxiety are likely to avoid the threatening stimulus (MacLeod & Mathews, 1988).

The present study involved training rats to perform a 3-choice serial reaction time task in response to both appetitive and fearful stimuli. The appetitive stimulus was a signal light to which correct responses were rewarded with water, and the fearful stimulus was another signal light to which misses and omissions were punished via a loud burst of white noise. A third signal light was also present, where incorrect responses were punished mildly. Since signals were presented for a very brief period of time, the accurate detection of these signals was assumed to be a valid measure of attention., A heightened accuracy for one of the signals over another would therefore be evidence for an attentional bias. Such a bias can then be compared to the subjects' anxiety levels, which we defined through performance on both an elevated plus maze and open field, two measures that are well established means for assessing anxiety in rats (Schmitt and Heimke, 1998).

This study attempts to provide a better understanding of the phenomenon of attentional bias by attempting to create an animal model that parallels the evidence of current human research. Since animals can be studied and manipulated more extensively than humans, this animal model could aid drug development and clinical therapy, as well

as offer insight into the attentional processes involved in threat detection, especially with regards to anxiety.

Methods

*Subjects***.** Seven adult Long-Evans rats (Harlan, Sprague-Dawley, Indianapolis, IN) were housed individually in a 12 hour light/dark cycle (lights on at 6:00 am and lights off at 6:00 pm) and had unlimited access to food and water until the beginning of the experiment. All subjects were allowed to acclimate to the vivarium for one week before any training or testing began; all testing occurred during the light phase. Each subject was given 15 minutes of free access to water in their home cage immediately after testing.

Apparatus. All training and testing occurred in standard operant chambers (28 cm length x 21 cm width x 27 cm height) located inside light and sound attenuating shells (64 cm x 41 cm x 41 cm). The front panel of each chamber contained three signal lights (2.8 W) centered 6 cm above three response levers. Each response lever was located 7 cm above a grid floor, and a water dispenser that delivered a single drop of water (40 ml) into a recessed water port (5 cm width x 3 cm depth x 5 cm height). The operant chambers were illuminated by a houselight (2.8 W) located 5 cm above the central signal light on the front panel, and were controlled by computers interfaced with hardware and software developed by Med Associates.

Anxiety Testing. The animals' basal anxiety level was defined by their performance during 3 ten minute sessions in both an elevated plus maze and an open field arena. The plus maze consisted of four perpendicular arms extending 2 feet from the center, forming a plus shape and elevated 4 ft above the floor; the sides of two opposite

arms were walled with Plexiglas, and the other two were open. A camera was mounted above the center to record the number of entries into each arm, as well as time spent within each arm. The open field arena was a 2 foot by 2 foot square box of Plexiglas, with a 4x3 matrix drawn on its base. Time spent in corners, sides, and the center was recorded by a camera mounted above the center. Each animal began in the center of the plus maze or open field arena and were randomly assigned to face a certain direction. The plus maze and open field arena were rotated between trials to prevent biases towards any specific direction or stimuli within the testing room. Anxiety testing took place in a well-lit room without the presence of the experimenter. All recordings were scored by an experimenter blind to the experimental conditions.

Training. Animals were first shaped in a three-lever paradigm under a FR-1 schedule until they pressed each lever at least 35 times in one hour for three consecutive days. The animals were then trained to discriminate between 3 signal lights. One of the signal lights was randomly lit for an indefinite period of time until the animal pressed the lever directly beneath the light, at which point a drop of water was dispensed as a reward; another signal light was randomly lit after a variable intertrial interval (ITI) which lasted 12 ± 3 s from lever press to the onset of the next signal. After reaching a criterion of at least 100 correct hits in one hour for three consecutive days, the duration of the signal lights was reduced to 1 s. Animals were rewarded for correct responses made either while the signal light was illuminated or during a 2 s response window. Correction trials were now incorporated into training sessions, in which misses and omissions resulted in a repetition of the signal type, until either a correct response or 5 repetitions occurred. Sessions lasted 36 minutes, as did all subsequent programs. Once the animals reached a

criterion of >70% correct responses and <20% omissions for each lever, they were moved to the final training program, where the duration of the signals varied between 50, 100, and 200 ms. Correction trials were used until the animals reached criterion, which was originally >70% correct responses and <20% omissions for each lever, but due to a ceiling effect, the animals were unable to reach this accuracy rate, and so it was lowered to >50% accuracy for each lever. Once correction trials were dropped, the animals continued to perform the final training program for 10 additional sessions, which was defined as 'baseline testing.' The animals then proceeded to the final testing program for 20 sessions, which is illustrated in Figure 2. For 4 of the animals, misses and omissions during left signal trials were punished with 5 s of aversive white noise (80 dB). This occurred with misses and omissions during right signal trials for the remaining 3 animals. For all subjects, misses and omissions during center trials were punished with 0.1 s white noise (80 dB). Correct responses to all signals were rewarded with water.

Statistical Methods. Elevated plus maze data were converted into anxiety scores as follows: (time spent in closed arms) / (time spent in open arms), and higher scores were defined as higher levels of anxiety. Open field data were converted into anxiety scores as follows: (time spent in corners) / (total time), with higher scores indicating higher anxiety levels. Baseline and testing data were analyzed using Signal Detection Theory (SDT), which assumes the existence of 2 intersecting normal distributions that represent the presence and absence of a signal. The distance between their means, which is labeled d', describes the discriminability of the signal, and equals Z(H) + Z(1-FA), where Z is a normalized score, H is the probability of a hit, and FA is the probability of a false alarm (signal is absent but the subject reports signal present). Also, there exists a

criterion, above which the subject reports the presence of a signal. This index, which is labeled β, describes the subject's readiness, or bias, in reporting the presence of a signal, and equals $e^{-(Z(H)^2-Z(1-FA)^2)/2}$. A low β reflects a bias to report the presence of a signal, while a high β reflects a bias to report the absence of a signal. Changes in d' and β from baseline were calculated and correlated with anxiety scores. The testing data were divided between the first 10 and second 10 sessions to abstract learning effects.

Results

During the first elevated plus maze session, no animal explored the open arms, and so the data were excluded. Because this session took place only 14 days after their arrival in the vivarium and before any training had taken place, it was assumed that they had not yet acclimated to their new environment; the data from the first open field session, which occurred the following day, was also excluded for consistency. The mean anxiety scores for the subsequent elevated plus maze sessions ranged from 0.52 to 0.73, and the mean anxiety scores from the open field sessions ranged from 0.38 to 0.73. The anxiety measures correlated negatively, yielding a Pearson correlation of -0.49 (Figure 3).

The group accuracies for baseline, first 10, and second 10 testing sessions are shown in Figure 4. The mean accuracy for nonpunished signal trials dropped substantially from baseline. However, the accuracies for both the center and punished signal trials were preserved. The group reaction times are illustrated in Figure 5. During the first 10 testing sessions, reaction times for each signal type increased by 70 ± 10 ms

above baseline, but dropped by 30 ± 10 ms below baseline during the second 10 testing days.

SDT analysis revealed that the subjects' criteria (β) for each signal type changed after testing. For the punished signal, the mean β of the group decreased by 0.10 after the first 10 testing sessions and increased by 0.19 after the second 10 sessions. The individual changes in β correlated strongly and negatively with elevated plus maze scores ($r_{1st\ 10}$ = -0.52, $r_{2nd\ 10}$ = -0.88), depicted in Figure 6. Thus, the most anxious subjects lowered their criteria after testing, enabling higher hit rates and/or higher false alarm rates. However, the changes in β correlated positively with open field scores ($r_{1st\ 10}$ = 0.46, $r_{2nd\ 10}$ = 0.49). Figure 7 illustrates these correlations, which suggest that highly anxious animals raised their criteria, reducing their accuracy and/or false alarm rates.

The mean β for the center signal decreased by 0.05 after the first 10 sessions, and then increased by 0.48 after the second 10 sessions. Elevated plus maze scores correlated negatively with the first 10 sessions ($r_{1st\ 10}$ = -0.54), and minimally with the second 10 sessions ($r_{2nd\ 10}$ =0.20). Open field scores also correlated weakly with changes in β ($r_{1st\ 10}$ = -0.14, $r_{2nd\ 10}$ = 0.35).

Subjects' discriminability (d') did not markedly change from baseline. The difference between the mean d' during baseline and the first 10 testing sessions was 0.06, and 0.02 during the second 10 testing sessions. Furthermore, the changes in d' after testing varied little between subjects.

The mean number of times in which subjects were punished with white noise was also analyzed and correlated with changes in β. Figure 8 illustrates the mild correlation between punishment and β, which were 0.57 during the first 10 sessions and 0.30 during

the second 10 sessions. However, punishment did not correlate with either anxiety measure: elevated plus maze ($r_{1st\ 10} = 0.05$, $r_{2nd\ 10} = 0.05$), open field ($r_{1st\ 10} = -0.12$, $r_{2nd\ 10} = -0.20$).

Discussion

Unfortunately, the two measures of anxiety used in this study correlated negatively with one another. One explanation is that the open field arena was walled with transparent Plexiglas. A typical open field arena has opaque or translucent walls, creating a contrast between exploration within the open center and the more secure sides and corners. Thus, time spent in the corners may not be a valid assessment of exploratory worry. On the other hand, the walls of the elevated plus maze were opaque and much closer resembled the standard.

The group exhibited a sharp drop in accuracy from baseline during nonpunished signal trials, while the accuracies for center and punished signal trials were preserved. This reflects the tendency of increased false alarms towards the center and punished levers. The increased reaction times during the first 10 sessions suggest an initial freezing response due to fear; after the animals learned the new payoff matrix, their reaction times decreased, as evidenced by the second 10 sessions. However, the changes in reaction time varied little between signal types, though we expected the subjects to hasten their responses to punished signals.

Nonetheless, the elevated plus maze data correlated very strongly with changes in criteria (β) during the second 10 testing sessions ($r = -0.88$), which signifies that 77.4% of

the variance is accounted for by elevated plus maze scores. Furthermore, the correlation between β and punishment was weak (r = 0.30), suggesting that the bias was not due to varying levels of exposure to punishment, which might enhance learning. This indicates that the methodology presented in this study may be applicable to the study of the attentional bias related to anxiety.

In order to make this methodology more soundly reflect the human condition, state anxiety should also be accounted for. This could be accomplished by administering anxiolytics or anxiogenics to the animals. According to the model by Williams et al., 1996 (Figure 1), low state anxiety should narrow the difference between low and high trait anxious subjects, eliminating the bias in the high trait anxious subjects who exhibit it. Inversely, under high state anxiety, the difference between low and high trait anxious subjects should broaden, with the latter displaying a solid bias for threat cues.

The present study possesses a few limitations. First, only seven subjects were used. Additional subjects could greatly improve statistical power and broaden correlational elements. Second, human research has revealed that the bias only occurs when multiple stimuli compete for attentional resources; anxious individuals do not detect lone threat cues faster than controls (Mathews & Milroy, 1994). The current method could be altered to resemble the dot probe detection task, such that two cues are presented simultaneously, followed by a signal to which the subject must respond. However, one could argue that because the locations of the signal lights compete for the animals' attentional resources, the bias results from a tendency to commit these resources to one of those locations over the others.

If the present methodology can be confirmed to be a sound animal model for anxiety-related attentional bias, it could be applied to a variety of future studies. For example, electrophysiological studies might clarify the relationship between signal detection and fear appraisal, and thus shed some light on the biological substrate of anxiety. Lesion and drug studies could also aid in the development of such knowledge. Even if an attentional bias for threats plays only a small factor in the development and maintenance of anxiety disorders, there is certainly a relationship, and a methodology similar to the present study could provide details that human research cannot.

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Figure Captions

Figure 1. Williams et al. (1997) model of attentional bias. The ADM determines how threatening a stimulus is, and state anxiety increases its output. If the threat value of a stimulus is high enough, the RAM is activated, causing high-trait anxious individuals to attend to the threat. However, the RAM does not cause low-trait anxious individuals to be biased towards the threat.

Figure 2. Schematic of the testing procedure. Circles represent signal lights, and parallelograms represent response levers. Each panel denotes one of the three signal trials, and the consequences of the various response possibilities are displayed below each parallelogram.

Figure 3. Correlation between the anxiety measures. The open field and elevated plus maze score correlated negatively.

Figure 4. Mean accuracies across the group. While the accuracies during nonpunished signal trials dropped substantially, the accuracies during the other trial types were preserved. N = nonpunished signal trials; C = center signal trials; P = punished signal trials.

Figure 5. Mean reaction times across the group. The reaction times for each signal type first increased, then decreased following testing. N = nonpunished signal trials; C = center signal trials; P = punished signal trials.

*Figure 6***.** The correlation between elevated plus maze scores and changes in β. During the first 10 and second 10 sessions, Δβ correlated negatively with elevated plus maze scores.

Figure 7. The correlation between open field scores and changes in β. During the first 10 and second 10 sessions, Δβ correlated positively with open field scores.

Figure 8. The correlation between mean punishment frequencies and changes in β. During the first 10 and second 10 sessions, Δβ correlated positively with the mean punishment frequencies.

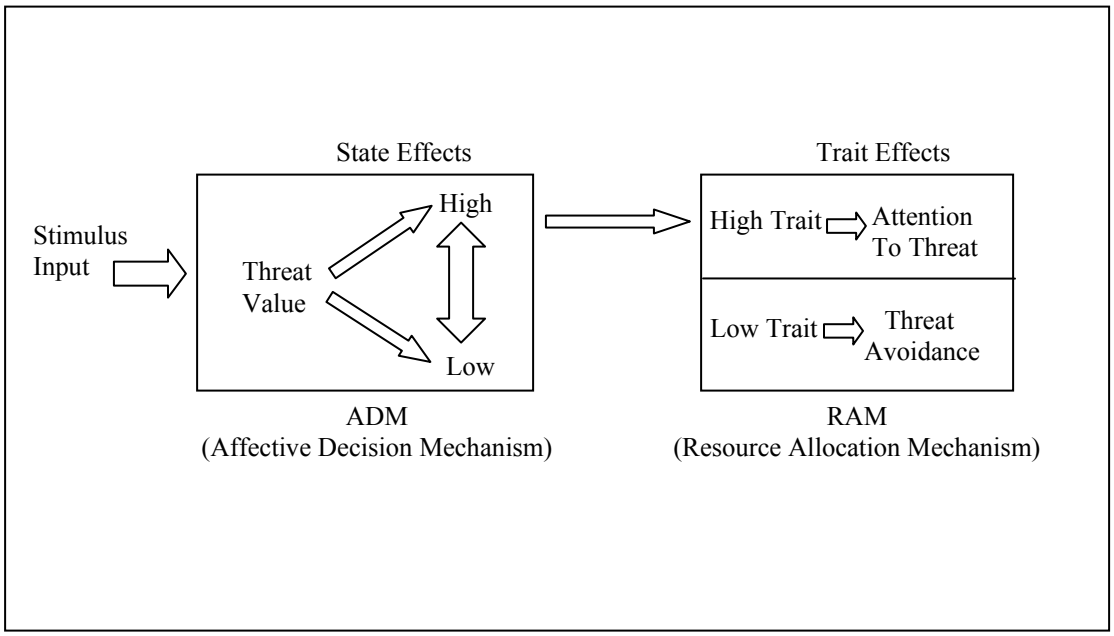
State Effects
Trait Effects
Stimulus
Input
Threat
Value
High
Low
High Trait
Attention
To Threat
Low Trait
Threat
Avoidance
ADM
(Affective Decision Mechanism)
RAM
(Resource Allocation Mechanism)

Figure 1.

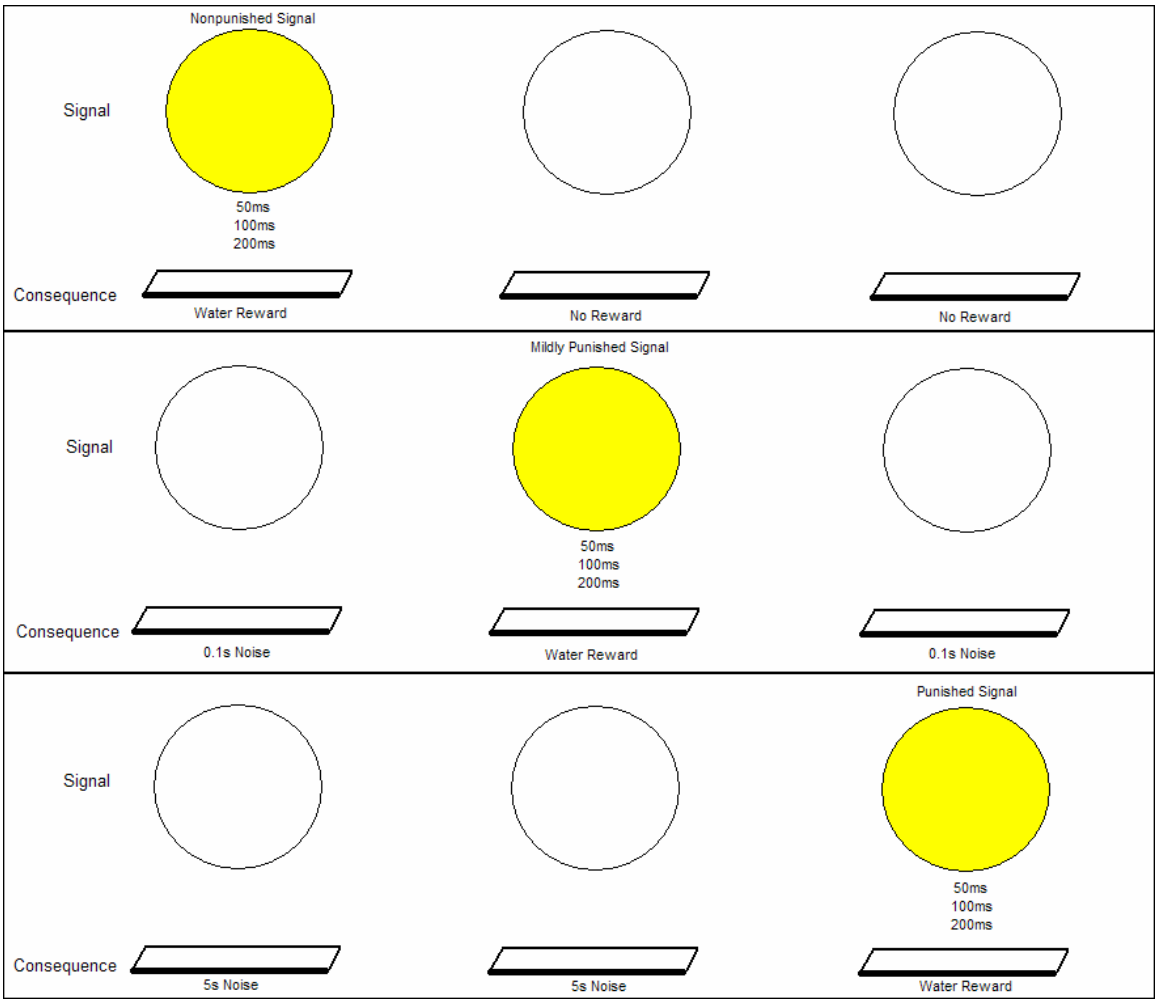
Nonpunished Signal
Signal
50ms
100ms
200ms
Consequence
Water Reward
No Reward
No Reward
Mildly Punished Signal
Signal
50ms
100ms
200ms
Consequence
0.1s Noise
Water Reward
0.1s Noise
Punished Signal
Signal
50ms
100ms
200ms
Consequence
5s Noise
5s Noise
Water Reward

Figure 2.

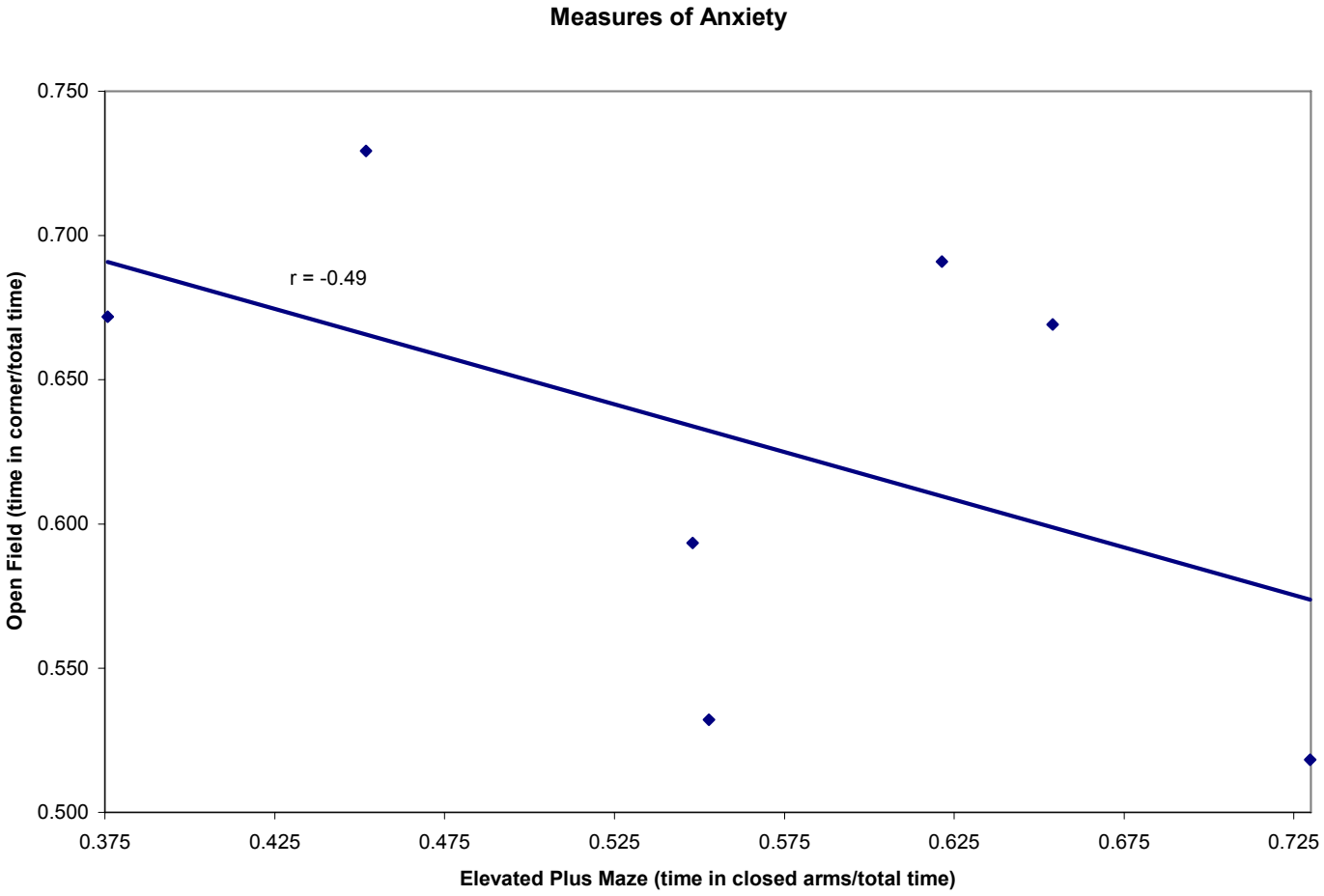
Measures of Anxiety
0.750
0.700
0.650
0.600
0.550
0.500
r = -0.49
Open Field (time in corner/total time)
0.375
0.425
0.475
0.525
0.575
0.625
0.675
0.725
Elevated Plus Maze (time in closed arms/total time)

Figure 3.

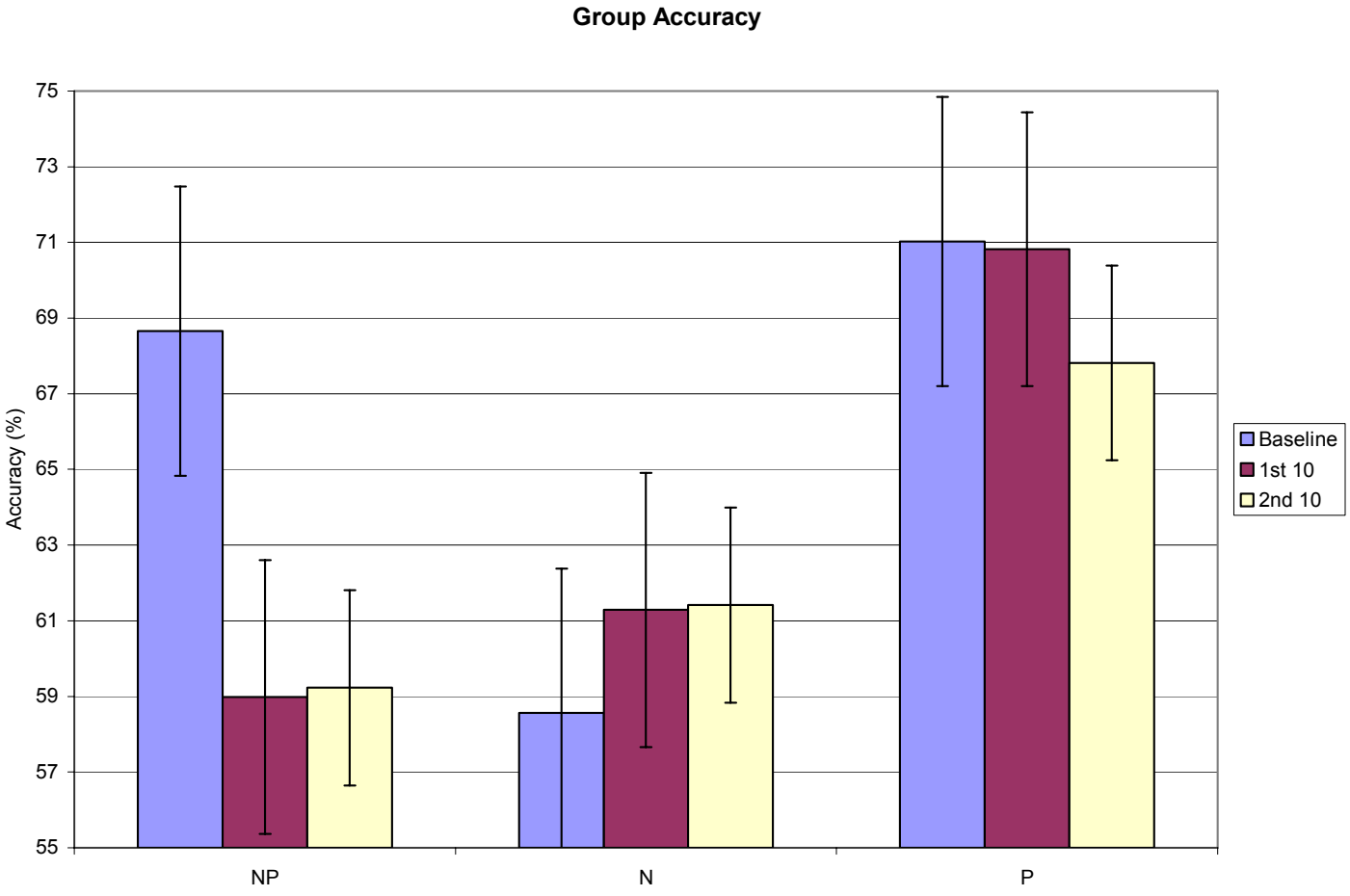
Group Accuracy
Accuracy (%)
75
73
71
69
67
65
63
61
59
57
55
NP
N
P
Baseline
1st 10
2nd 10

Figure 4.

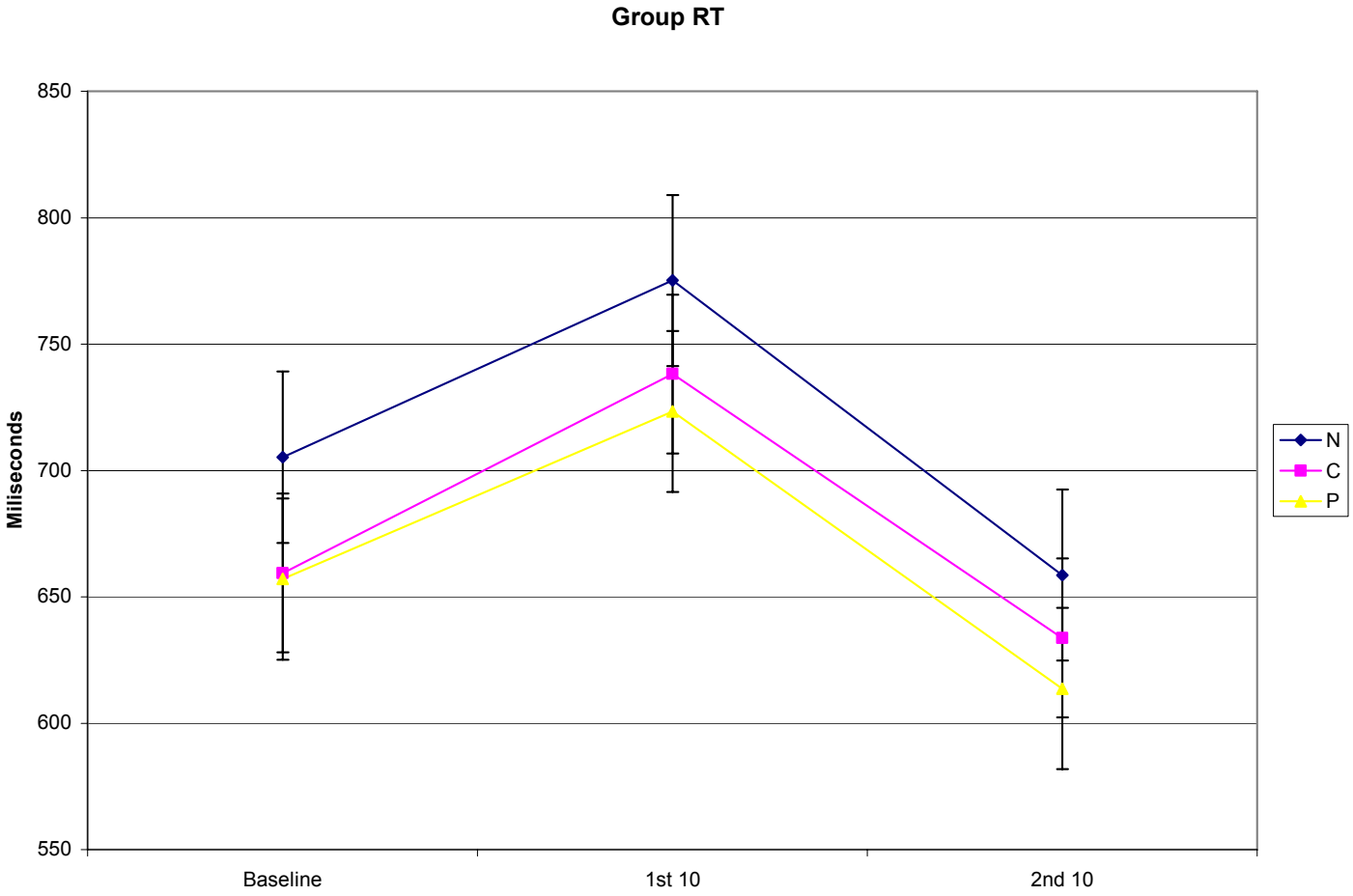
Group RT
Miliseconds
850
800
750
700
650
600
550
Baseline
1st 10
2nd 10
N
C
P

Figure 5.

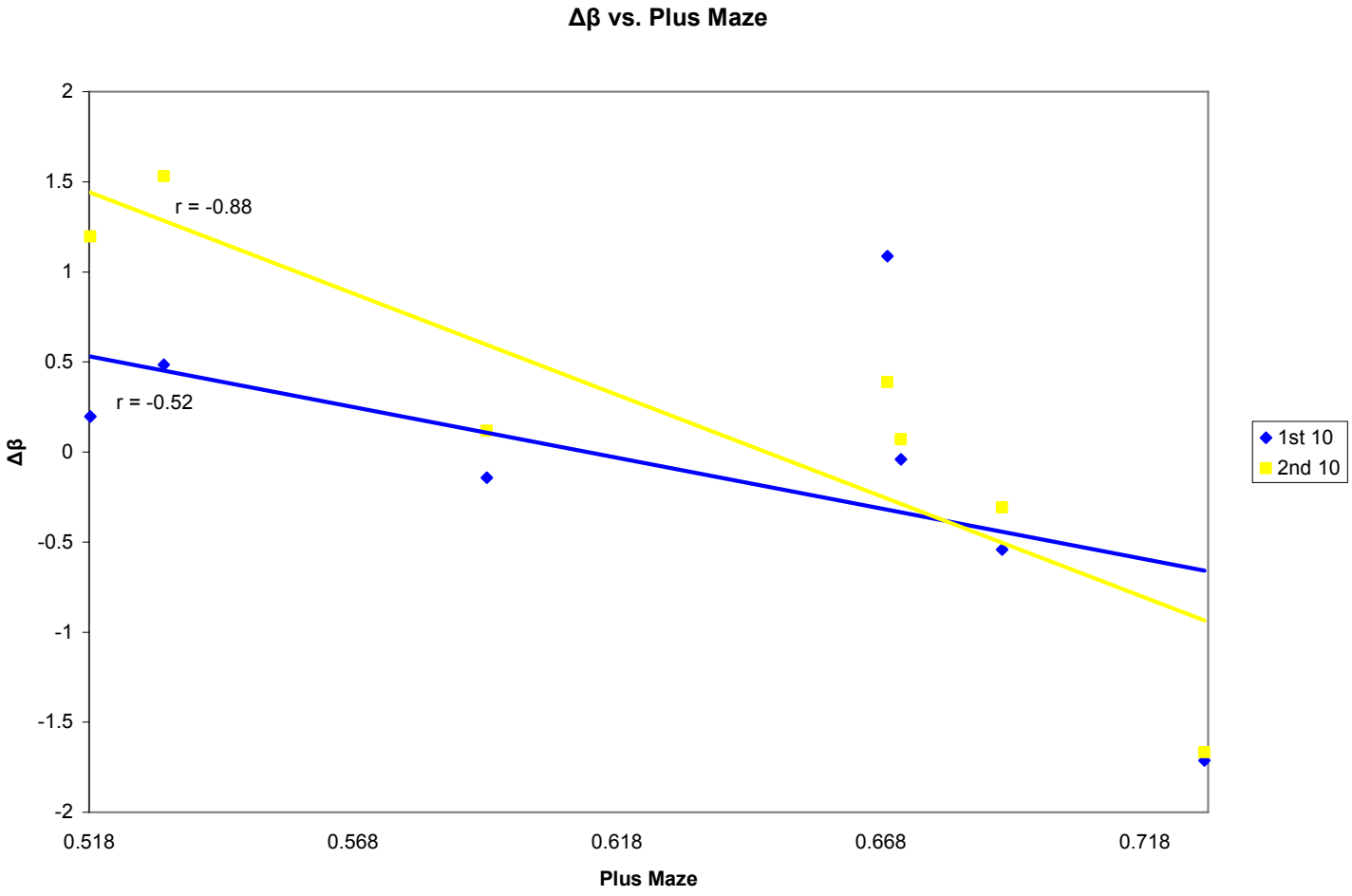
Δβ vs. Plus Maze
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
Δβ
r = -0.88
r = -0.52
0.518
0.568
0.618
0.668
0.718
Plus Maze
1st 10
2nd 10

Figure 6.

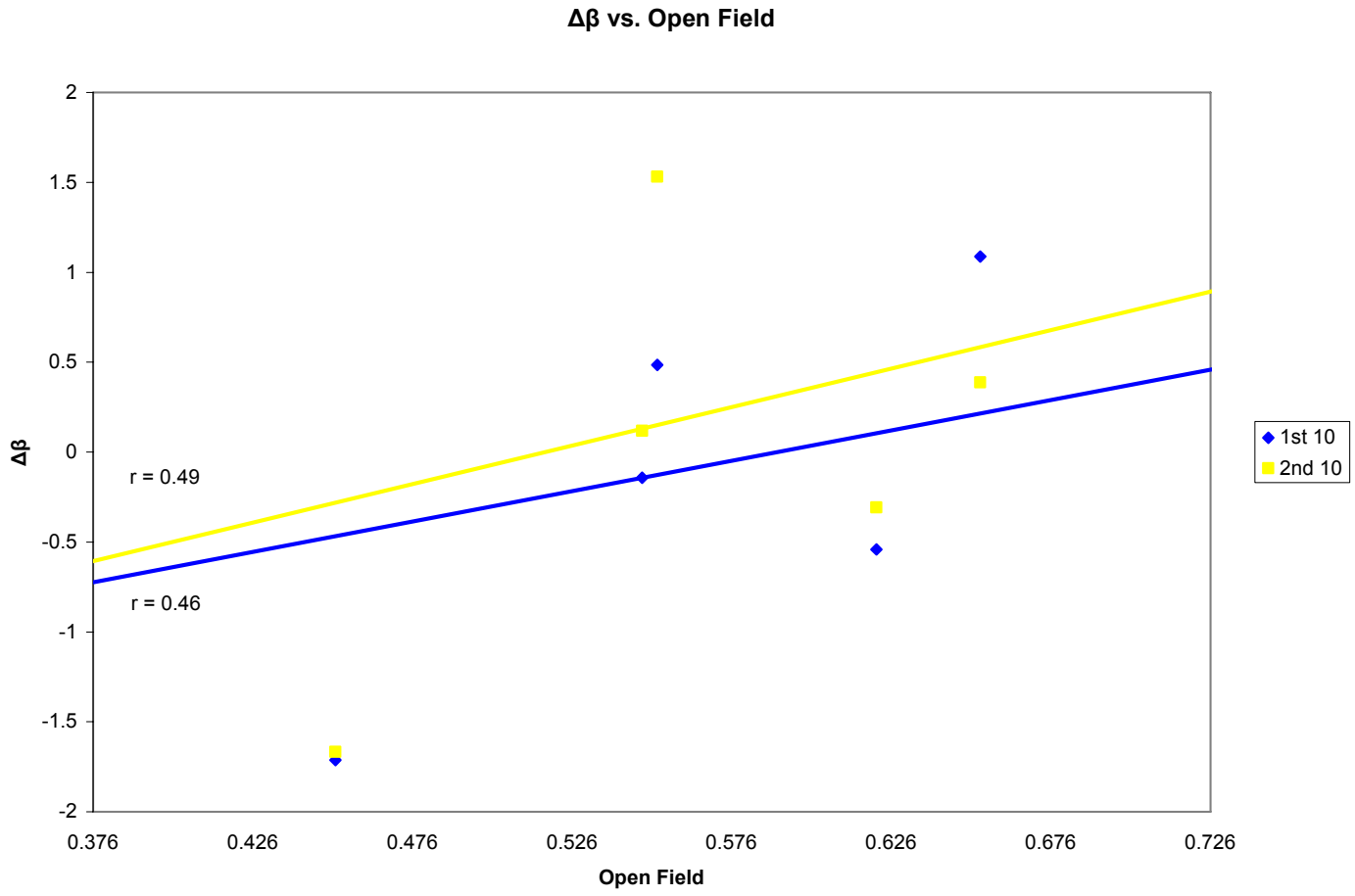
Δβ vs. Open Field
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
Δβ
r = 0.49
r = 0.46
0.376
0.426
0.476
0.526
0.576
0.626
0.676
0.726
Open Field
1st 10
2nd 10

Figure 7.

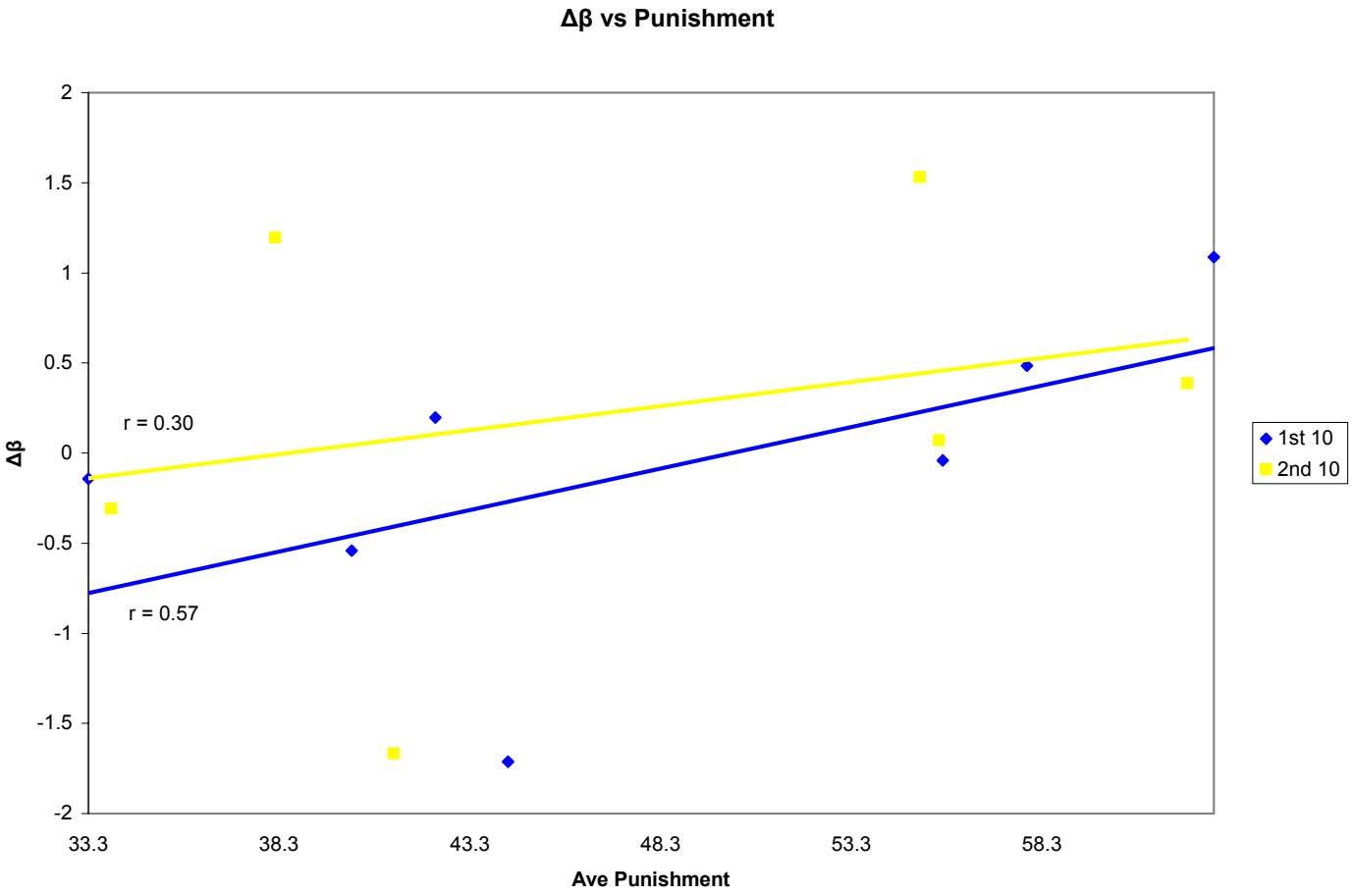
Δβ vs Punishment
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
Δβ
r = 0.30
r = 0.57
33.3
38.3
43.3
48.3
53.3
58.3
Ave Punishment
1st 10
2nd 10

Figure 8.